J. Dairy Sci. 88:1741–1744

Short Communication: Ca^{2+}-Adenosine Triphosphatase Protein Expression in the Mammary Gland of Periparturient Cows

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ABSTRACT

The objectives of this study were to measure the changes in protein expression of the mammary Ca^{2+}-ATPases during the periparturient period and to determine whether Ca^{2+}-ATPase protein expression in the mammary gland is related to milk fever (MF) development. Abundance of Ca^{2+}-ATPase in mammary tissue and milk fat globule membranes was determined by Western blotting. The secretory pathway Ca^{2+}-ATPase was elevated prepartum in mammary tissue from cows that developed MF compared with non-MF cows.
(**Key words:** Ca^{2+}-adenosine triphosphatase, milk fever, milk fat globule membrane, mammary gland)

Abbreviation key: MF = milk fever, **MFGM** = milk fat globule membrane, **MG** = mammary gland, **PMCA** = plasma membrane Ca^{2+}-ATPase (The number following PMCA and SPCA refers to the specific isoform. The first lowercase letter following the number in PMCA refers to the splice site C splice form, and the next lowercase letter refers to the splice site A splice form). **SPCA** = secretory pathway Ca^{2+}-ATPase.

Research on milk fever (**MF**) has historically focused on methods to control intestinal Ca absorption and bone Ca resorption in the periparturient cow (Goff and Horst, 2003; Horst et al., 2003). The potential role that mammary gland (**MG**) Ca storage and transport has on the development of MF has been ignored despite the primary role of the MG in MF development (Goff et al., 2002). The objectives of this study were to measure MG and milk fat globule membrane (**MFGM**) expression of the major mammary Ca^{2+}-ATPase proteins in the periparturient period and examine Ca^{2+}-ATPases protein expression in normal and MF cows.

Three weeks prepartum, 15 Jersey cows were fed a diet that promoted MF (Goff et al., 2002). Mammary gland biopsies were collected from 12 cows on d –7, 0, 7, 14, with d 0 representing calving day. Because of the uncertainty of the time of calving, the d –7 samples ranged from –4 to –12 d. After calving, cows were fed a normal lactation diet (Goff et al., 2002). Blood samples were collected to monitor plasma Ca and plasma $1,25(OH)_2D_3$ concentrations (Reinhardt et al., 1984). Cows were classified MF cows according to their clinical signs and plasma Ca levels. Mammary gland, MFGM, and bovine brain membranes were prepared as previously described (Reinhardt et al., 2000). Membrane preparations were stored at –70°C. Proteins were determined using the BioRad Protein Assay Kit. The Ca^{2+}-ATPase protein expression in the MG and MFGM was determined by Western blotting as described by Reinhardt et al. (2000). Antiplasma membrane Ca^{2+}-ATPase 2 and 4 (**PMCA2**, **PMCA4**) and secretory pathway Ca^{2+}-ATPase 1 (**SPCA1**) antibodies were described previously (Reinhardt et al., 2000, 2004a,b). One lane in every gel was loaded with a constant amount of brain membrane to serve as a standard. The results from image analysis for the blots were normalized to the brain microsomal membrane lane and expressed in arbitrary units.

Data were analyzed by repeated measures using the PROC MIXED procedure of SAS (SAS Inst., Inc., Cary, NC). The model included the fixed effects of time and MF status, time by MF interaction, the random effect of cows nested within MF status, and the residual error. For each variable analyzed, cow nested within MF status was subjected to 3 covariance structures; compound symmetry, autoregressive order 1, and unstructured covariance. The covariance that resulted in the Akaike's information criterion closest to zero was used, which was autoregressive order 1. Means and standard errors of the means are reported for all data. Means separation was conducted by the Tukey-Kramer option in SAS.

Seven of 15 cows developed MF. Plasma Ca concentration decreased in MF cows just before ($P < 0.05$) and at parturition ($P < 0.001$) (Figure 1A) compared with non-MF cows. Plasma $1,25\text{-}(OH)_2D_3$ concentrations were elevated on d 1 prepartum ($P < 0.05$) through d 2 ($P < 0.001$) of lactation in MF cows (Figure 2B) compared with non-MF cows. The expression of Ca^{2+}-ATPase pro-

Received August 26, 2004.
Accepted November 29, 2004.
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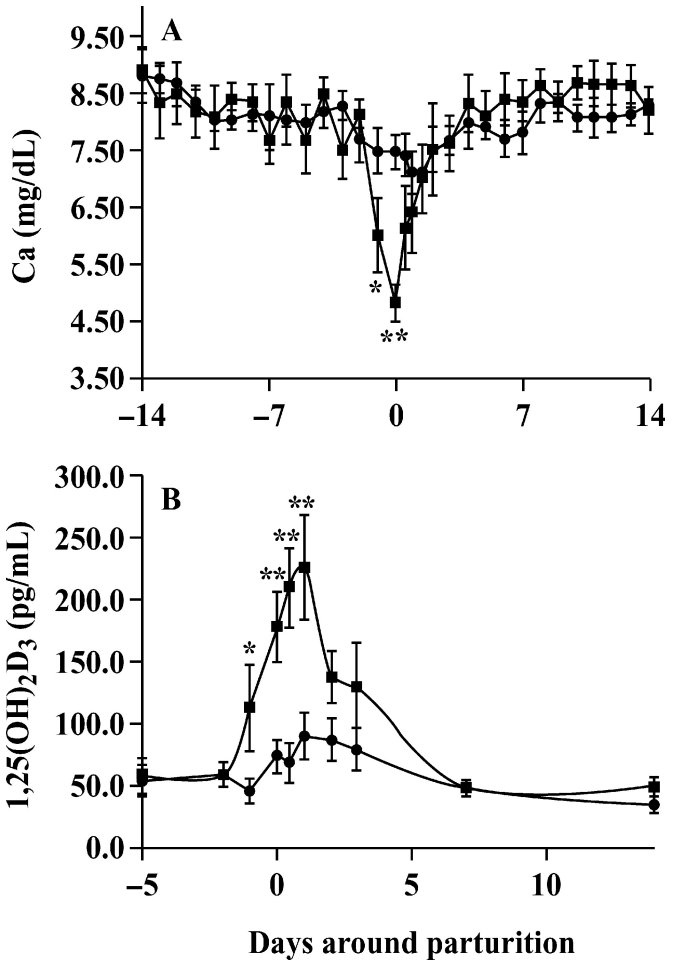

Figure 1. Plasma calcium (panel A) and plasma $1,25(OH)_2D_3$ (panel B) concentrations in milk fever (MF) cows (n = 7; ■) and non-MF cows (n = 8; ●) during the periparturient period. Significantly different from non-MF cows: $^*P < 0.05$; $^{**}P < 0.001$.

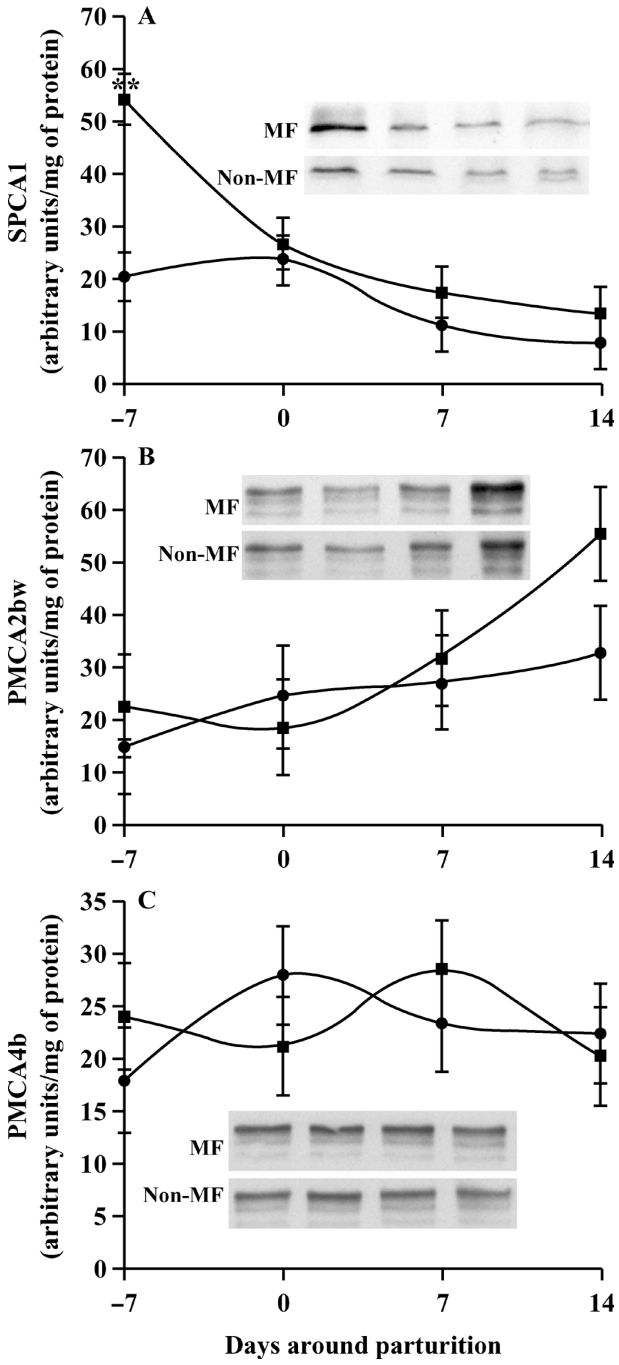

Figure 2. Calcium pump expression in mammary gland biopsies during the periparturient period in milk fever (MF) cows (n = 6; ■) and non-MF cows (n = 6; ●). Panel A: SPCA1, panel B: PMCA2bw; and panel C: PMCA4b. PMCA = plasma membrane Ca^{2+}-ATPase (The number following PMCA and SPCA refers to the specific isoform. The first lowercase letter following the number in PMCA refers to the splice site C splice form, and the next lowercase letter refers to the splice site A splice form). SPCA = secretory pathway Ca^{2+}-ATPase. Representative Western blots for SPCA1, PMCA2bw, and PMCA4b from a cow with MF and a non-MF cow are presented as insets in the figures. Significantly different from non-MF cows: $^{**}P < 0.001$.

teins in the MG was measured in MG biopsies (Figure 2). There was an effect of MF status on SPCA1 expression ($P < 0.05$), and the MF by time interaction was significant ($P < 0.001$). Milk fever cows expressed more SPCA1 ($P < 0.001$) prepartum than non-MF cows (Figure 2A). The SPCA1 expression was not different between MF and non-MF cows from parturition through d 14 of lactation and declined from prepartum levels ($P < 0.001$). The PMCA2bw expression (Figure 2B) was not different between MF and non-MF cows. The MF by time interaction was not significant. Expression of PMCA2bw did increase with time and was elevated ($P < 0.05$) on d 14 of lactation compared with d −7. Expression of PMCA4b (Figure 2C) remained constant and was not different between MF and non-MF cows. The interaction was not significant. Expression of PMCA2bw in MFGM (Figure 3A) was not affected by MF status. The MF by time interaction was not signifi-

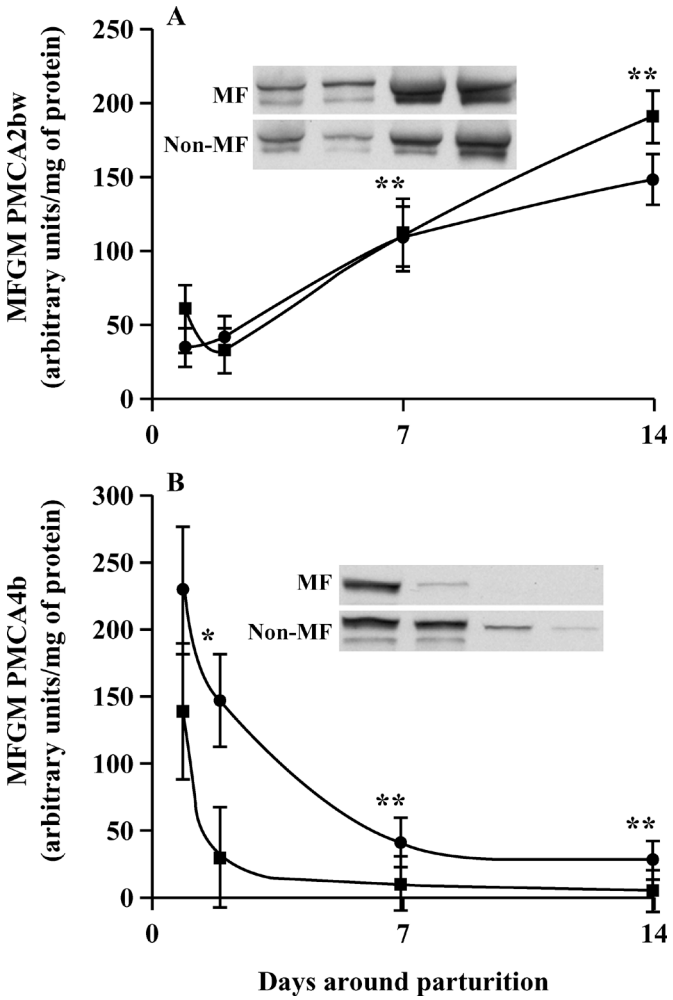

Figure 3. Calcium pump expression in milk fat globule membranes (MFGM) during lactation in milk fever (MF) cows (n = 7; ■) and non-MF cows (n = 8; ●). Panel A: PMCA2bw; panel B: PMCA4b. PMCA = plasma membrane Ca^{2+}-ATPase (The number following PMCA refers to the specific isoform. The first lowercase letter following the number in PMCA refers to the splice site C splice form, and the next lowercase letter refers to the splice site A splice form). Representative Western blots for PMCA2bw and PMCA4b from a cow with MF and a non-MF cow are presented as insets in the figures. Significantly different from lactation d 1 for all cows: *$P < 0.05$; **$P < 0.001$.

cant. Expression of PMCA2bw in MFGM, however, increased ($P < 0.001$) in all cows by d 7 and 14 of lactation. Expression of PMCA4b in MFGM was lower ($P < 0.05$) in cows that developed MF (Figure 3A), but the interaction was not significant. In all cows, PMCA4b expression (Figure 3B) in MFGM declined as lactation proceeded ($P < 0.05$).

Although there are numerous theories regarding the causes of MF, one fact is indisputable: the MG is the key. Mastectomy totally eliminates blood Ca declines at parturition in dairy cows (Goff et al., 2002). The lactating mammary gland is the site of significant Ca storage and transport (Swanson et al., 1956; Baumrucker, 1978). The early work of Swanson et al. (1956) showed that cows prone to MF had larger mammary Ca storage pools. Based on the data of Swanson et al. (1956), the Golgi location of SPCA1 (Reinhardt et al., 2004a), and the timing of SPCA1 expression prepartum (Reinhardt and Horst, 1999; Reinhardt et al., 2000), we hypothesized that SPCA1 expression prepartum may be more highly expressed in MF cows. The resulting higher rate of Ca flow into the Golgi would result in greater loss of body Ca to the MG at a critical time and explain in part the degree of hypocalcemia experienced by MF cows. The data show that SPCA1 protein expression in the MG is significantly ($P < 0.001$) higher in prepartum cows that develop MF (Figure 2A). At parturition, SPCA1 expression in the MF cows was equal to that of the non-MF cows, but its higher expression prepartum may be just enough Ca stress to upset Ca homeostasis, thus leading to MF. In the MFGM, PMCA4b was significantly lower in MF cows (Figure 3B). For this, we have no explanation, as the role of PMCA4b in the MG has yet to be explored. It has been speculated the PMCA4b may be important to mammary development (Reinhardt et al., 2000), but PMCA4 has been shown to have few critical functions (Okunade et al., 2004).

The precise metabolic lesions responsible for MF development are still being debated and are likely a complex interacting network of Ca stresses that, when added together, result in the development of this disease. This study provides data that indirectly suggest that increased prepartum MG Ca storage may contribute to the development of MF. Cows that develop MF express significantly more SPCA1 in their MG prepartum. The SPCA1 is a Golgi Ca pump responsible for loading Ca into the Golgi. Therefore, these data provide new and novel insights into potential additional mechanisms associated with MF development. In addition, data on the protein expression of the Ca pump (PMCA2bw) responsible for Ca transport into milk (Reinhardt et al., 2004b), SPCA1, and PMCA4b are presented for the first time in the cow.

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